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Supporting Information

SARS-CoV-2 N protein induces acute kidney injury via Smad3-dependent G1 cell cycle arrest mechanism

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Supplementary Information

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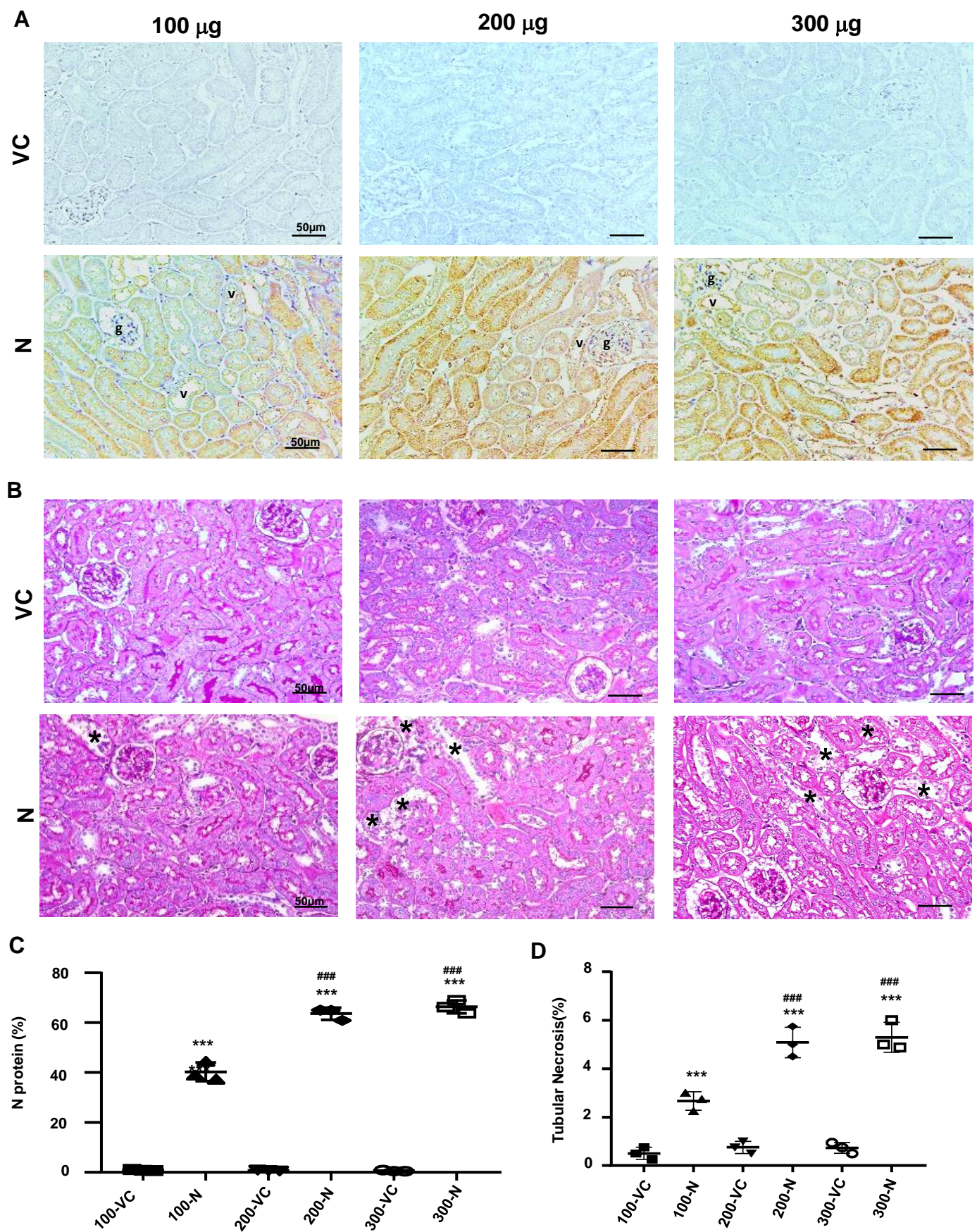**Co-correspondence: Jianguo Wu, jwu898@jnu.edu.cn; Xueqing Yu, yuxq@mail.sysu.edu.cn

Figure S1. Ultrasound-microbubble-meditated kidney-specifically SARS-CoV-2 N protein (N) expression induces tubular necrosis in a dose-dependent manner. (A) N protein immunostaining; **(B)** PAS-staining; **(C)** Semi-quantitative analysis of N protein; **(D)** Semi-quantitative analysis of tubular necrosis (*). Note that SARS-CoV-2 N protein plasmid transfer results in overexpression of N protein and tubular necrosis in a dose-dependent manner, peaking at 200 μg/mouse. Each dot represents one mouse and data are expressed as the mean ± SEM for groups of 3 mice. ***p<0.001 versus vector control (VC); ###p<0.001 versus SARS-CoV-2 N-expressing plasmid at 100 μg/mouse (100-N). g, glomerulus; v, vascular cells. Scale bar=50 μm.
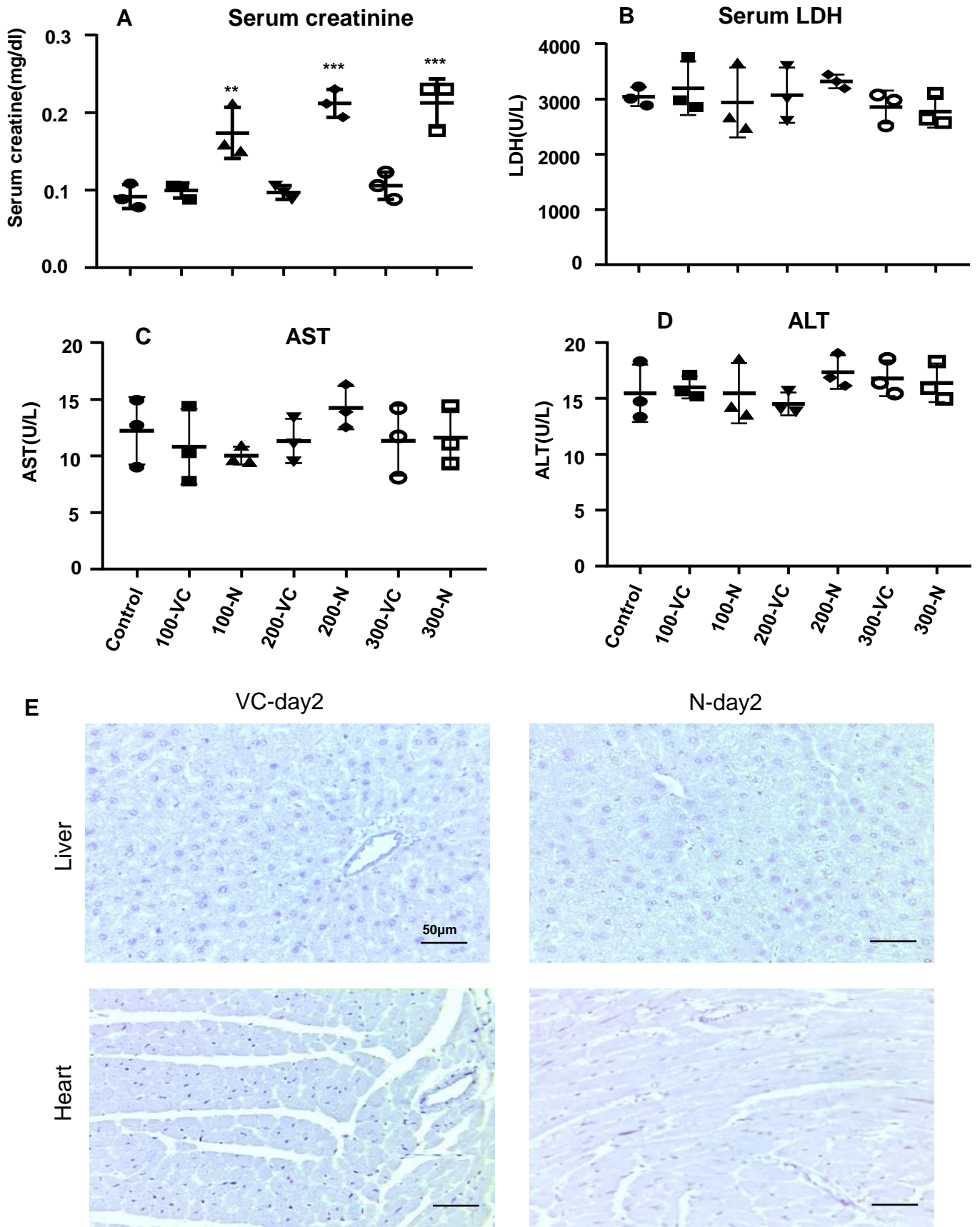
Figure S2. Ultrasound-microbubble-meditated kidney-specifically SARS-CoV-2 N protein (N) expression results in a significant increase in serum creatine in a dose-dependent manner without systemic toxicity. (A) Serum creatine; **(B)** serum LDH; **(C)** AST; **(D)** ALT; **(E)** immunohistochemical staining shows that expression of SARS-CoV-2 N protein is not detectable in both liver and hear tissues after ultrasound-microbubble-mediated kidney-specifically SARS-CoV-2 N transfection. Each dot represents one mouse and data are expressed as the mean ± SEM for groups of 3 mice.**p<0.01, ***p<0.001 versus vector control (VC).
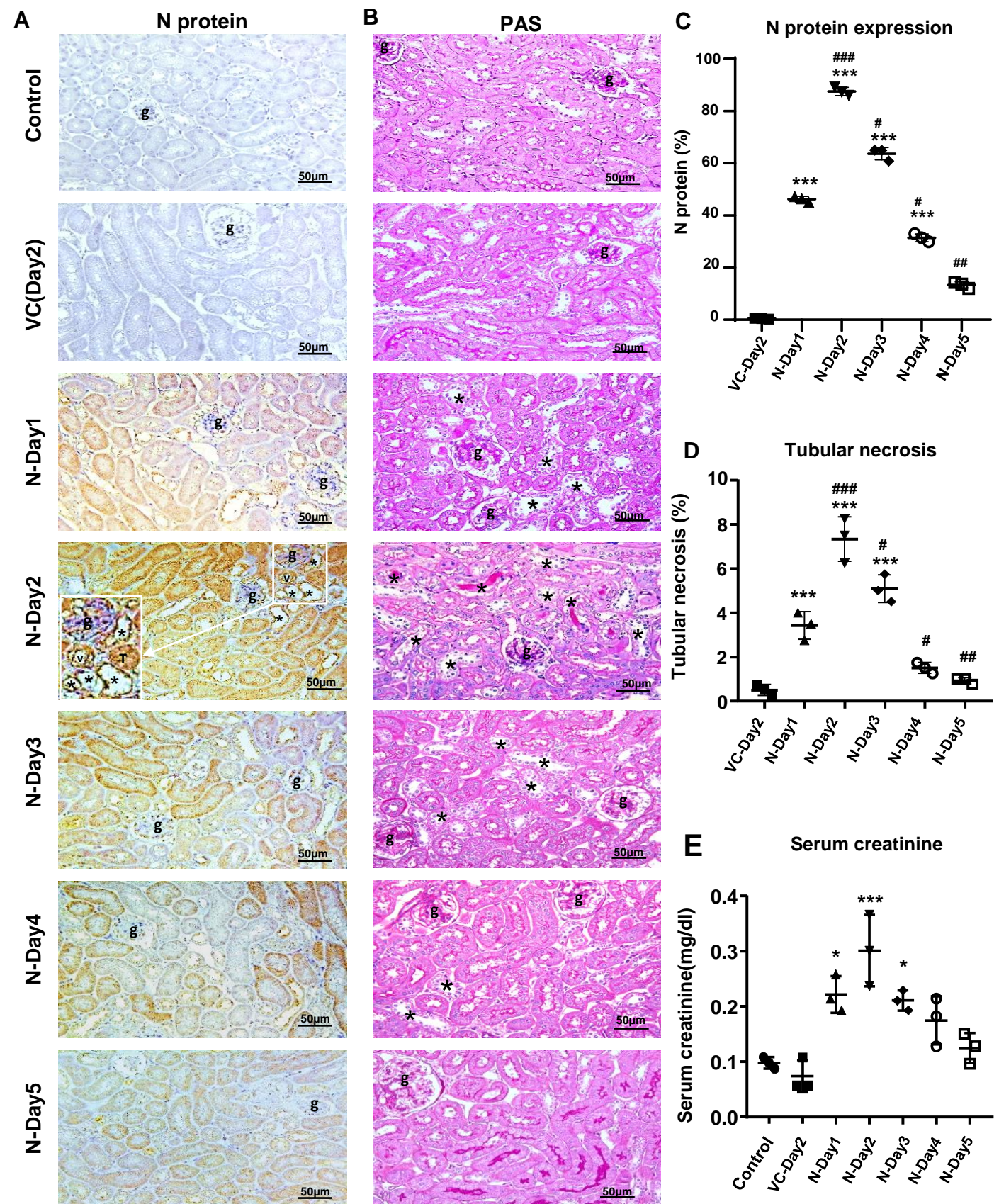
Figure S3. Kidney-specifically overexpressing SARS-CoV-2 N protein (N) causes AKI in a time-dependent manner, peaking at day 2 after the N gene transfer. (A) N protein immunostaining; **(B)** PAS-staining; **(C)** Semi-quantitative analysis of N protein; **(D)** Semi-quantitative analysis of tubular necrosis (*); **(E)** Serum levels of creatinine. Note that kidney-specifically SARS-CoV-2 N-expressing plasmid transfer results in overexpression of N protein primarily in tubular cells with tubular necrosis (*) as well as in glomerular (g) and vascular (v) cells (see inserted picture at day 2) in a time-dependent manner, peaking at day 2. Each dot represents one mouse and data are expressed as the mean ± SEM for groups of 3 mice. *p<0.05, ***p<0.001 versus vector control (VC); #p<0.05, ##p<0.01, ###p<0.001 versus SARS-CoV-2 N-expressing plasmid at day 1 (N-day 1). g, glomerulus. Scale bar=50 μm.
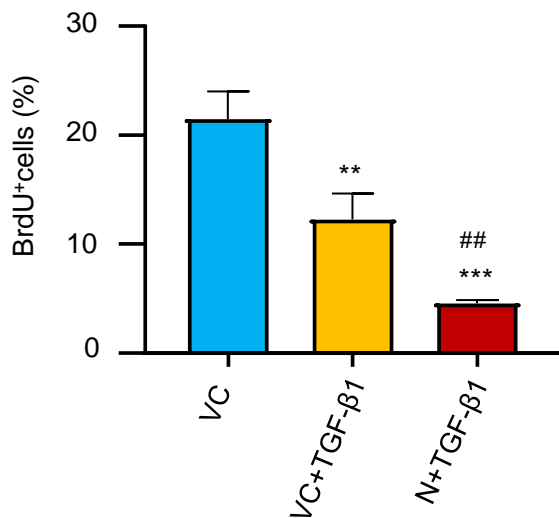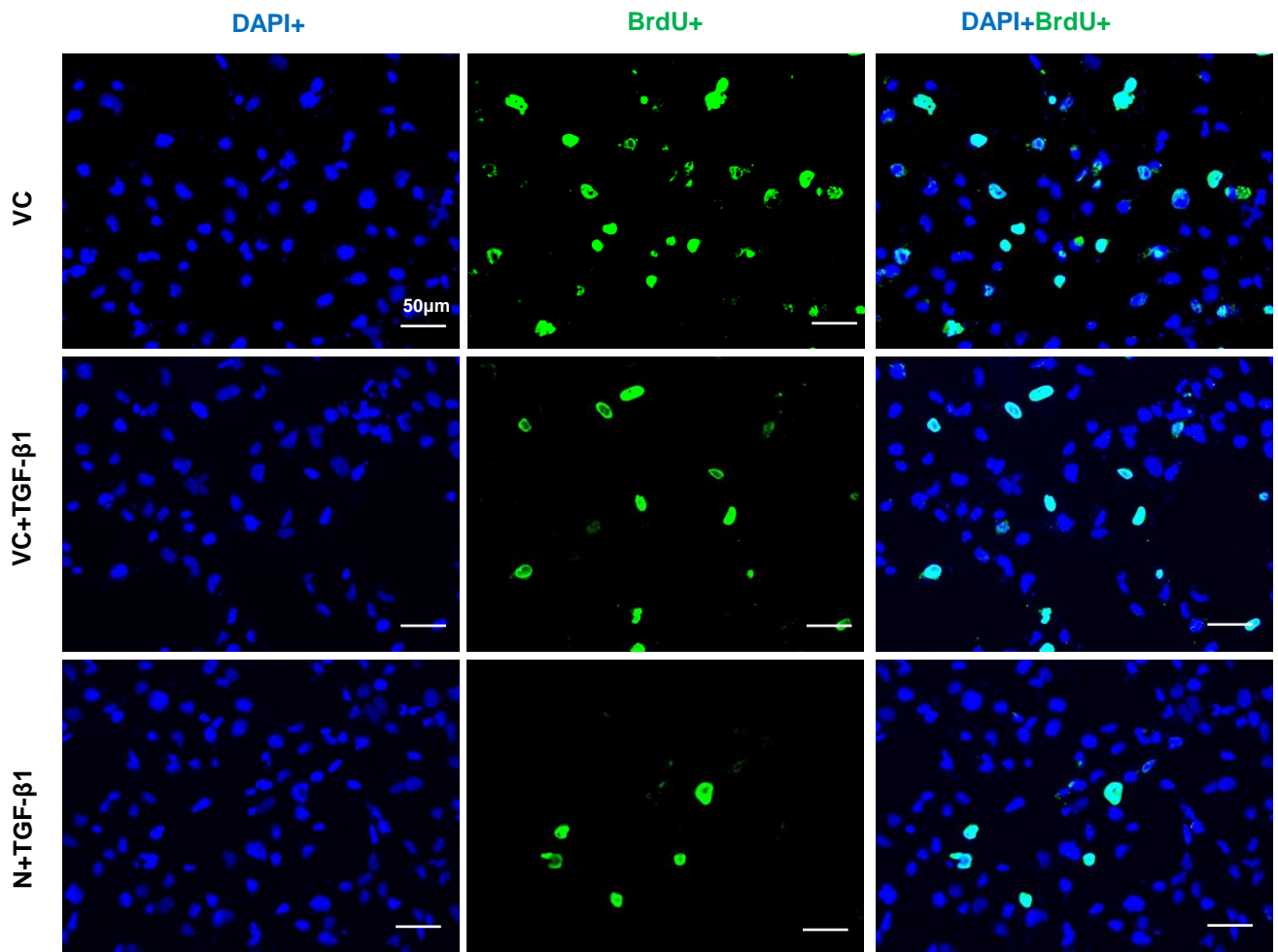
Figure S4. Immunofluorescence detects that overexpressing SARS-CoV-2 N protein (N) largely promotes TGF-β1-induced inhibition of S-phase cell cycle in human TECs (HK-2) in vitro. Note that under normal conditions, HK-2 cells are highly proliferative as demonstrated by many cells entering into the S-phase of cell cycle, which is inhibited by addition of TGF-β1 (5ng/ml) and becomes worsen by co-overexpressing SARS-CoV-2 N protein. Each bar represents the mean ± SEM for 3 independent experiments.**p<0.01, ***p<0.001 versus vector control (VC); ## p<0.001 versus VC + TGF-β1. Scale bar=50 μm.
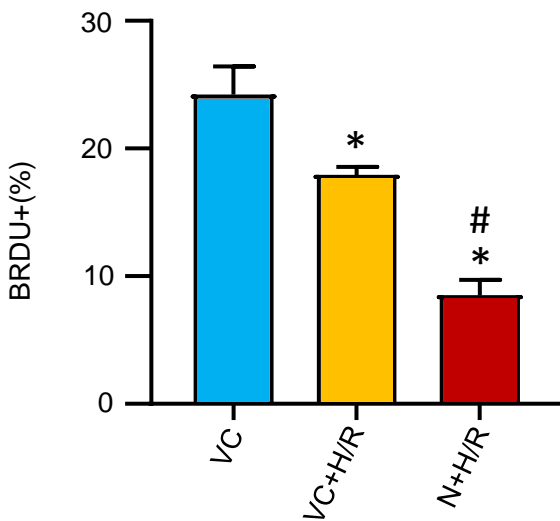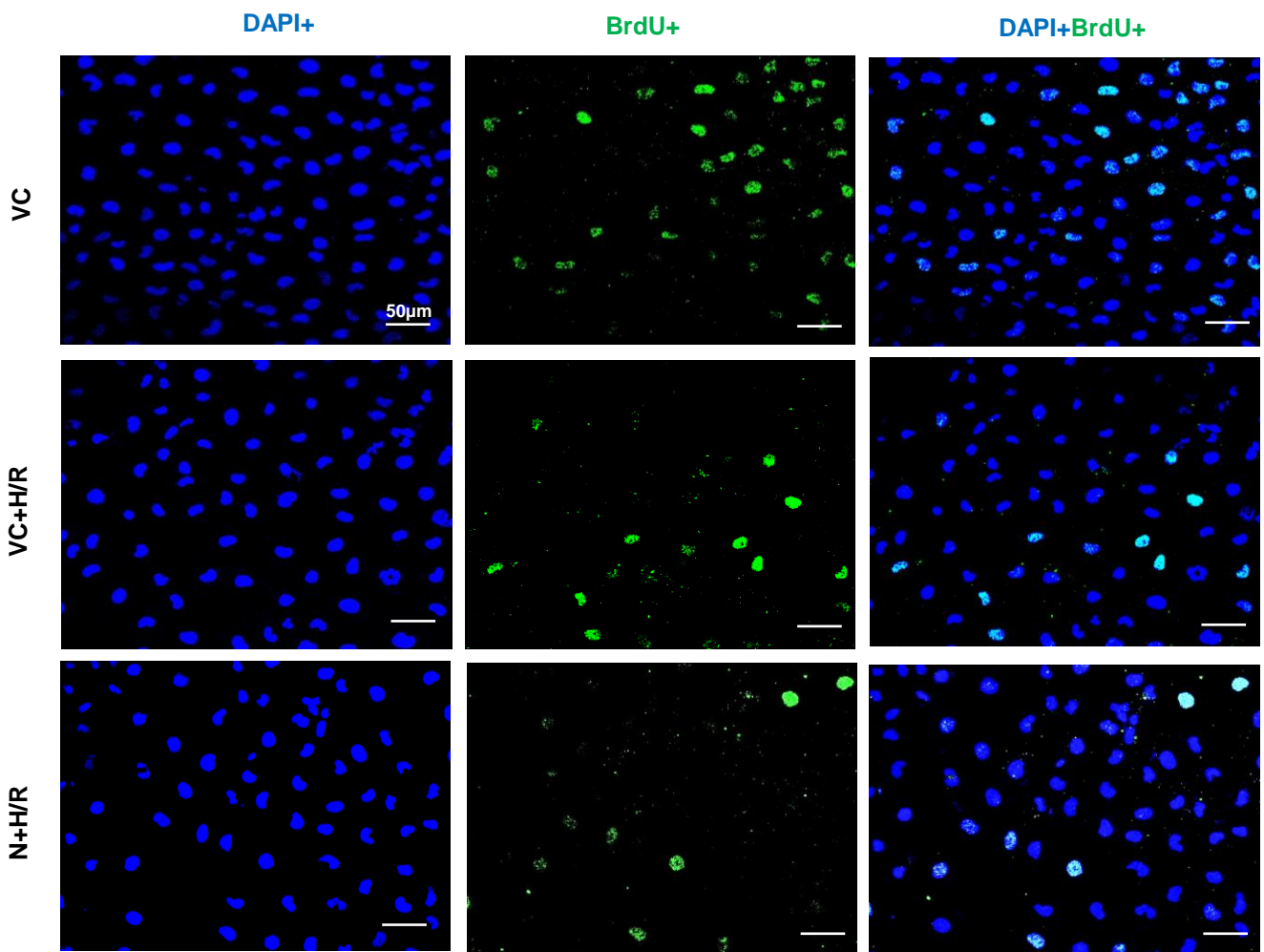
Figure S5. Immunofluorescence detects that overexpressing SARS-CoV-2 N protein (N) largely promotes hypoxia/reoxygenation-induced inhibition of S-phase cell cycle in human TECs (HK-2) in vitro.
Note that under normal conditions, HK-2 cells are highly proliferative as demonstrated by many cells entering into the S-phase of cell cycle, which is inhibited by hypoxia/reoxygenation injury and becomes worsen by overexpressing SARS-CoV-2 N protein. Each bar represents the mean $\pm$ SEM for 3 independent experiments. .*$p<0.05$ versus vector control (VC); # $p<0.005$ versus VC +H/R. Scale bar=50 μ m.
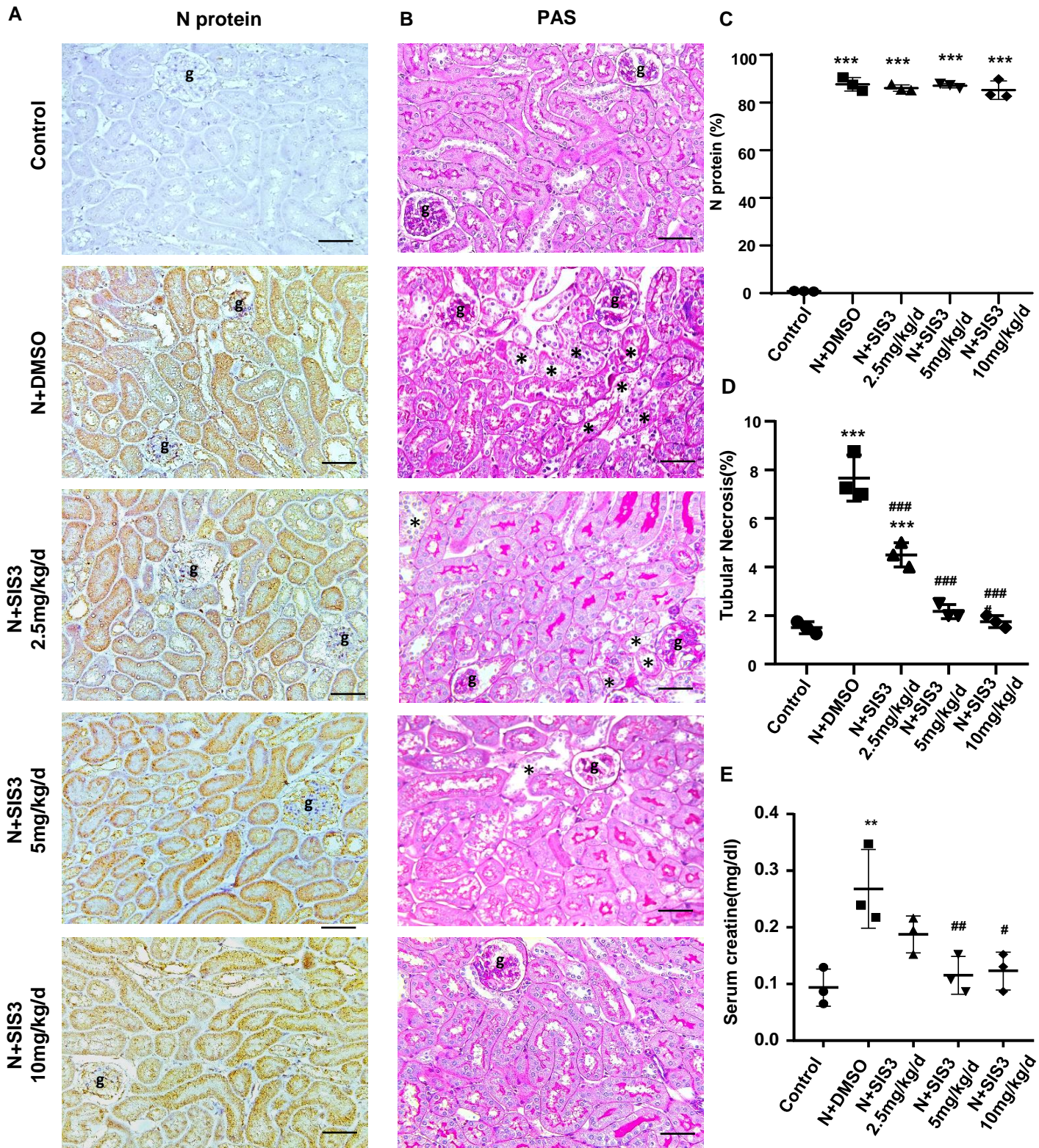
Figure S6. Treatment with SIS3 dose-dependently inhibits SARS-CoV-2 N protein-induced AKI without altering expression of N protein in the kidneys. (A) SARS-CoV-2 N protein immunostaining; **(B)** PAS-staining for detection of tubular necrosis (*); **(C)** Semi-quantitation of the N protein expression in the kidney; **(D)** Semi-quantitation of renal tubular necrosis; **(E)** Serum levels of creatinine. Each dot represents one mouse and data are expressed as the mean ± SEM for groups of 3 mice. **p<0.01, ***p<0.001 versus vector control; #p<0.05, ##p<0.01, ###p<0.001 versus overexpression of SARS-CoV-2 N + DMSO-control treatment (N+DMSO). g, glomerulus. Scale bar=50 μm.
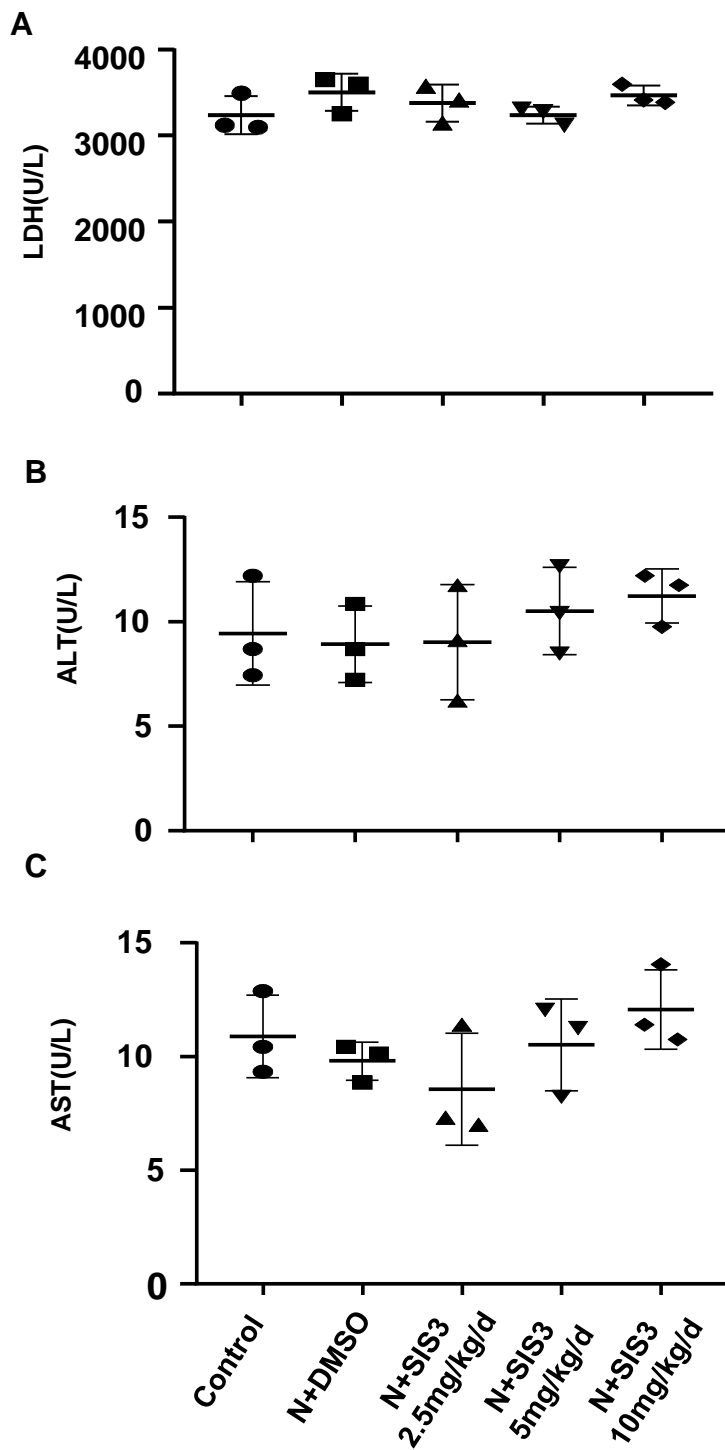
Figure S7. Systemic toxicity of SIS3 in mice with SARS-CoV-2 N protein-induced AKI. (A) Serum levels of LDH release; (**B**) Serum levels of ALT; **(C)** Serum levels of AST. Each dot represents one mouse and data are expressed as the mean ± SEM for groups of 3 mice.
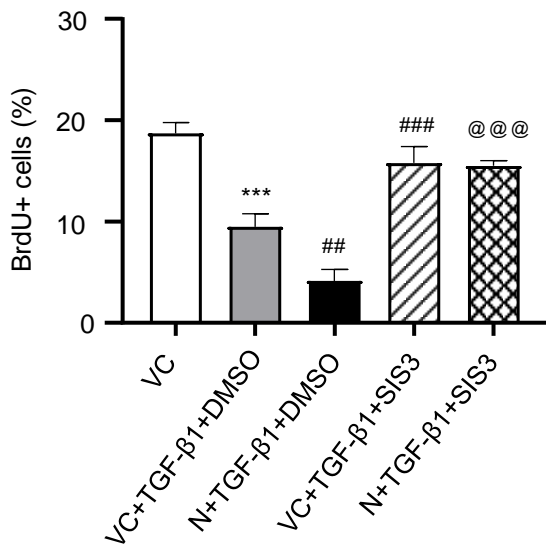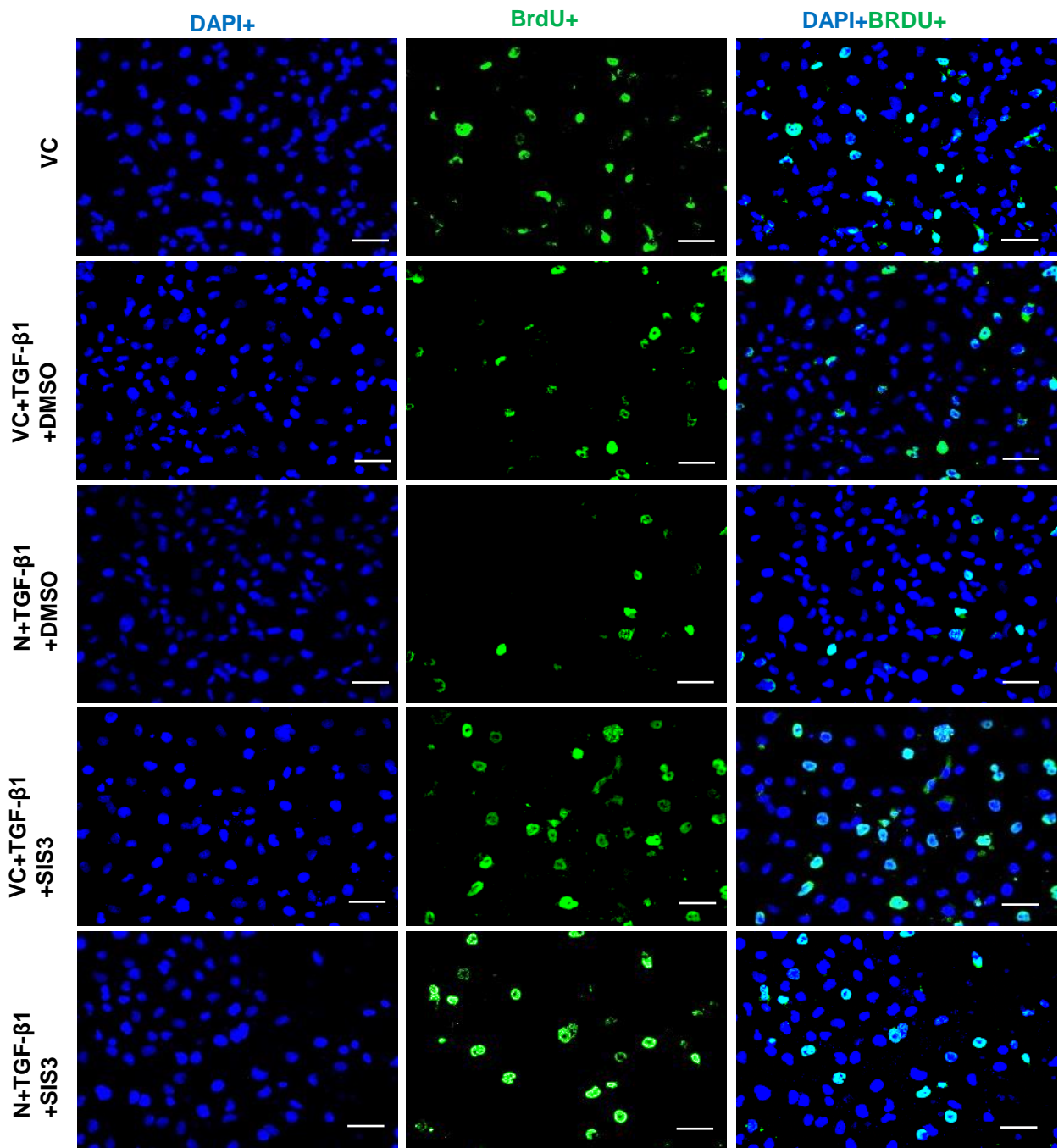
Figure S8. Blockade of Smad3 reverses SARS-CoV-2 N-induced inhibition of cell cycle progression at the S-phase in HK-2 cells in vitro. Immunofluorescence detects that overexpression of SARS-CoV-2 N protein (N) can severely impair TEC proliferation by blocking the S-phase cell cycle progression , which is virtually blocked with a Smad3 inhibitor SIS3. Note that SIS3 treatment also block TGF-β1-induced inhibition of S-phase cell cycle in HK-2 cells. Each bar represents the mean ± SEM for groups of 3 independent experiments. *p<0.05, ***p<0.001 versus vector control (VC); ##p<0.01, ###p<0.001 versus VC+TGF-β1+DMSO; @@@ p<0.001 versus N+TGF-β1+DMSO. Scale bar=50 μm.